Super Hotstart Taq Polymerase

#P1201, 250U

Concentration: 5U/μl
Contents:

Super Hotstart Taq Polymerase (5U/μl)	50 μl
10× Hotstart Buffer (Mg^{2+} Plus)	1.25 ml

Store at -20°C

For research use only.

In total 2 vials.

Description

Super Hotstart Taq Polymerase is a hot-start polymerase with dual-antibody modification, which brings higher specificity by reducing non-specific products as the enzyme activity is temperature-dependent and is inhibited at room temperature. Its initial-denaturation time can be reduced to 1 minute. Super Hotstart Taq provides excellent specificity, sensitivity and yield. The reaction system can be prepared at room temperature. The amplification length and speed can reach to 5 kb (simple template) and 2min/kb (simple template up to 20s/kb) separately. Super Hotstart Taq has 5'-3' polymerase activity, but no 3'-5' exonuclease activity. The products of Super Hotstart Taq have overhanged dA at 3'-end.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Storage Buffer

200mM Tris-HCl, 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.5% Tween20, 50% (V/V) glycerol, 0.5% Triton X -100

10× Hotstart Buffer with Mg2+

50mM KCl, 100mM Tris-HCl, 200mM NH_4Cl, 20mM $MgCl_2$.

Applications

• High-specificity amplification & multiplex PCR: hot-start polymerase with antibody modification

Features

• Thermostable: half-life over 40 min at 95°C incubation
• Generates 3'-dA overhangs PCR products

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of *Super* Hotstart Taq Polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

Reagent	**50-µl rxn**	**Final conc.**
10× Hotstart Buffer (Mg^{2+} plus)	5 µl	1×
dNTPs (10mM each)	1 µl	0.2mM each
Primer I	variable	0.4-1µM [1]
Primer II	variable	0.4-1µM [1]
Super Hotstart Taq Polymerase	0.5-1.0 µl	2.5-5U/50 µl [2]
Template DNA	variable	10pg-1µg [3]
Nuclease-free water [4]	To 50 µl	-

[1] Recommended range of final primer concentration: 0.1-1µM. The concentration can be reduced when the specificity is poor, and the concentration can be increased when the efficiency is low.

[2] The amount of DNA polymerase is adjusted according to the difficulty of amplification of the target fragment.

[3] Recommended amounts of template DNA in a 50 µl reaction mix:

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

[4] Ultrapure nuclease-free water can be ordered separately (Cat. # : P9021/P9022/P9023).

2. Mix contents in tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94°C	2 minutes
25-35 Cycles	94°C 55°C [1] 72°C [2]	30 seconds 30 seconds Variable
Final Extension	72°C	5~10 minutes

[1] The annealing temperature should be set according to primers with lower Tm values.

[2] The optimal extension time is 2min/kb (up to 20s/kb for simple templates).

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.

Notes on Cycling Conditions

- Super Hotstart Taq Polymerase adopts improved antibody modification technology, so it relies on temperature to activate the polymerase activity, which can effectively inhibit non-specific bindings, and the reaction system can be configured at room temperature.
- The half-life of enzyme is >40 minutes at 95°C.
- Super Hotstart Taq Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Guidelines for Preventing Contamination of PCR Reaction

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 10U *Super* Hotstart Taq Polymerase with 1μg pBR322 DNA for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 10U *Super* Hotstart Taq Polymerase with 1μg digested DNA for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10U *Super* Hotstart Taq Polymerase with 1μg E.coli [3H]-RNA (40000cpm/μg) for 4 hours at

37°C and 70°C.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.